Phosphatidylcholine Biosynthesis as a Potential Target for Inhibition of Metabolism in Parasitic Nematodes

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Abstract: Parasitic nematodes are major causes of human, animal, and plant diseases worldwide. Although a number of therapeutics are available as treatments, reported resistance to certain anthelmintics, severe side-effects, or limited efficacy resulting from differences in the life cycles of target organisms underscore the need for the continued development of nematicidal compounds. Identifying biochemical targets that differ between the parasite and host species is essential for finding effective new molecules. The free-living nematode *Caenorhabditis elegans* serves as a useful model system for studying nematode biology and for analyzing the biochemistry of enzymes in potential target pathways. Providing a major component of cellular membranes, the core metabolic pathways of phosphatidylcholine synthesis in eukaryotes are well conserved; however, recent studies suggest that nematodes (and Plasmodia) use a different metabolic route to this phospholipid than mammals. In addition, phosphatidylcholine is a precursor in the production of glycoconjugates secreted by parasitic nematodes to avoid host immune responses. RNA-mediated interference experiments in *C. elegans* suggest that the enzymes of phosphatidylcholine biosynthesis are essential for nematode normal growth and development. Therefore, small molecule inhibitors of these enzymes may be valuable as medical, veterinary, and agricultural nematicides. This review examines the current state of knowledge of phosphatidylcholine biosynthesis in the model organism *C. elegans*.

Keywords: Phosphatidylcholine, phosphocholine, parasite, nematode, *Caenorhabditis elegans*, kinase, methyltransferase, cytidylyltransferase.

PARASITIC NEMATODES

The worldwide impact of parasites on human, animal, and plant life is profound with the array of parasites varying as widely as the range of species and environments found in nature. Helminths, including nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms), and protozoans, such as Plasmodia and trypanosomes, cause a variety of diseases in man, livestock, and crops. In particular, nematode parasites are responsible for a range of health, veterinary, and agricultural problems.

The number of infected individuals, animals, and crops and the amount of money spent combating these organisms, reflects the scope of damage caused by nematodes and the importance of controlling these parasites. In humans, *Ascaris lumbricoides* (intestinal roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* (hookworm) and *Ancylostoma duodenale* (hookworm) infect approximately one billion people [1]. The diseases caused by these parasites result in morbidity, blindness, anemia, intestinal disease, respiratory problems, and disfigurement of major organs and limbs [1-2]. Likewise, zoonotic parasites infect pets and livestock, reducing weight gain and milk production [3]. Global sales of veterinary anthelmintics are more than $1 billion annually with North America and Europe accounting for 60% of spending [3-4]; however, more than 70% of production animals are in developing nations where constant and seasonal exposure to parasites is most severe [4]. Estimates of the damage caused by nematode infections of different crops and horticultural plants indicate a ten to fifteen percent decrease in annual yields with an economic cost of $125 billion per year [4-5]. For example, soybean cyst nematode (*Heterodera glycines*) and root-knot nematodes (*Meloidogyne* sp.) cause $1 billion in damage to crops in the United States alone [6].

The existing medical and veterinary pharmacopoeia targeting nematode parasites is chemically varied [2, 7-13]. The benzimidazoles, including thiabendazole, mebendazole, and albendazole, target β-tubulin and are used for the treatment of intestinal roundworms (soil-transmitted *Ascaris*, hookworms, and *Trichuris* whipworms), tissue roundworms, and other intestinal tapeworms [2, 9]. Levamisole, an imidathiazole, is effective against roundworms and hookworms [12]. Ivermectin, a polyketide, is traditionally used as a potent broad spectrum anthelmintic of animal parasites and is now widely employed in combating the tissue nematode *Onchocerca volvulus*, which causes river blindness, and other nematodes (*Wuchereria bancrofti* and *Brugia malayi*) that cause lymphatic filariasis [10]. Diethylcarbamzine is also a common treatment for patients with lymphatic filariasis [13]. The primary impetus to develop new anthelmintics, much like the drive for novel antibiotics, is drug resistance. Currently, reports describe resistance to all compounds of the few anthelmintic classes available [8-9]. Although there are only a few reported cases of resistance to available drugs in humans, nematode resistance problems in livestock, including cattle, sheep, goats, and pigs, are severe and widespread [8-9].

In agriculture and animal husbandry, parasite management efforts are widely employed, especially where

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economics, government regulation, or grower preferences limit chemical or pesticide control methods [7, 14-16]. Breeding of crops for nematode resistance can be effective, but genetic sources of resistance are not available for many crops and the effect is not always permanent [16]. Application of chemicals, such as methyl bromide or fenamiphos (Nemacur), can be effective; however, most nematicides used in agriculture have serious safety and environmental issues [7]. Older compounds were derived from industrial by-products or broad-spectrum insecticides. For example, fenamiphos is an organophosphate pesticide that acts as a cholinesterase inhibitor. The lack of safe, specific molecules targeting plant parasitic nematodes leaves few control options for growers. Moreover, side-effects and limited efficacy resulting from differences in the life cycles of target organisms underscore the need for the continued development of nematicidal compounds and for basic research studying parasitic nematodes of humans, animals, and plants [11].

Given the time and cost investments for new therapeutics, efforts to identify novel targets and discover new anthelmintic or nematicidal molecules are necessary. A critical tool in this process is the free-living nematode *Caenorhabditis elegans*, which provides a tractable system for studying nematode biology and for analyzing the biochemistry of potential target pathways. Recent studies suggest that nematodes (and the protozoa *Plasmodium falciparum*, the causative agent of malaria) may use a metabolic route to phosphatidylcholine that differs from mammals. This review examines available information on phosphatidylcholine biosynthesis in *C. elegans*.

C. ELEGANS AND COMPARATIVE NEMATOLOGY

Sydney Brenner began exploring the value of *C. elegans* as a model system for developmental biology in the sixties and early seventies [17], culminating in the Nobel Prize for Physiology or Medicine in 2002, which was shared with H. Robert Horvitz and John E. Sulston, for elucidating how key genes regulate organ development and programmed cell death. Since these pioneering steps, numerous researchers have adopted *C. elegans* as a scientific tool.

Multiple properties make this organism an excellent biological system for the analysis of diseases and for drug discovery, as discussed in recent reviews [11,18-20]. The worms are easily cultured and molecular methods are well established and freely shared between *C. elegans* laboratories [21-22]. The complete developmental lineage of each cell in the organism is known [23]. Importantly, *C. elegans* was the first eukaryote with a fully sequenced genome (10^8 base pairs (bp)) [24]. On-going functional annotation of the genome continues through a consortium of scientists who maintain WormBase (www.wormbase.org), a database that provides functional annotation of gene/protein function, genetic data, mutant phenotypes, and expression/interaction data [25]. Moreover, multiple open-access resources for researchers working with *C. elegans* (and other nematodes) are available (Table **1**).

Another key feature of using *C. elegans* is the ability to perform RNA-mediated interference (RNAi) to analyze gene function [26-28]. In *C. elegans*, direct injection of a double-stranded RNA (dsRNA) encoding a portion of a target gene or feeding of the worms with bacteria expressing dsRNA results in post-transcriptional gene silencing by RNAi [27-28]. Unlike mammalian systems, in which small interfering RNA (siRNA) (21-23 bp) are used to reduce non-specific effects, induction of RNAi in *C. elegans* requires at minimum a 100 bp dsRNA with typical dsRNA lengths of 500 to 1500 bp routinely used. In addition, off-target effects associated with RNAi are minor in *C. elegans* compared to siRNA gene silencing [29]. RNAi is also a keystone in genome-wide chemical and genetic screens for the identification of phenotypes associated with silencing of specific genes [30-32]. Kamath *et al.* [30] demonstrated the adaptability of *C. elegans* to high-throughput screens by employing RNAi to identify mutant phenotypes for 1,722 genes out of 16,700 predicted genes inhibited.

Multiple pharmaceutical companies use *C. elegans* as a model organism for drug and target discovery (Table **1**). As

Table 1. Online Nematode Resources and Companies Using *C. Elegans* as a Drug Discovery Tool

Website Address	Description
www.wormbase.org	Wormbase: a *C. elegans* database
www.nematode.net	Nematode Genome Sequencing Center
www.nematodes.org	Nematode and neglected Genomes
www.wormbook.org	Wormbook: open-access *C. elegans* methods
www.celeganskoconsortium.omrf.org	Production of deletion alleles in *C. elegans*
www.cbs.umn.edu/CGC	Caenorhabditis Genetics Center (CGC)
www.axyspharm.net	Axys Pharmaceuticals
www.cambriabio.com	Cambria Biosciences
www.devgen.com	Devgen
www.divergence.com	Divergence, Inc.
www.exelixis.com	Exelixis, Inc.

a model nematode, *C. elegans* also provides a system for the analysis of molecular targets of anti-parasitic drugs and for evaluating the effect of chemical compounds [19-20]. For example, the molecular targets of levamisole (nicotinic acetylcholine receptor), aldicarb (acetylcholine esterase), benzimidazoles (β-tubulin), and ivermectin (glutamate-gated chloride channels) were all identified in this worm [11, 19-20,33]. Likewise, comparative genomic efforts are exploiting the wealth of data generated for this organism to identify potential new anthelmintic and nematicide targets.

Although basic information obtained through studies of *C. elegans* remains valuable, variation in lifecycle and growth environments of different parasitic nematode species requires greater understanding of parasite adaptations [4, 18]. Building on the foundation provided by *C. elegans*, large-scale sequencing and expressed-sequence tag (EST) surveys are in progress for a range of nematode parasites, including *B. malayi* (lymphatic filariasis), *O. volvulus* (river blindness), *Haemonchus contortus* (sheep barber pole worm), *Toxocara canis* (dog roundworm), *Litomosoides sigmodontis* (rodent filariasis), *N. americanus* (human hookworm), *Teladorsagia circumcincta* (sheep roundworm), *Trichuris muris* (mouse whipworm), *Ostertagia circumcincta* (sheep stomach worm), *A. suum* (pig intestinal roundworm), *A. lumbricoides* (large roundworm of human), *Meloidogyne hapla* (root-knot nematode), and *Trichinella spiralis* (human-infective muscle cell nematode) [34-36]. Moreover, the first steps towards gene silencing by RNAi in parasitic nematodes (*B. malayi* and *Nippostrongylus brasiliensis*) have been demonstrated [37-38]. The continued application of more resources and new methodologies to elucidate the differences between host and parasitic organisms promises the development of strategies for treating or controlling nematode parasites.

ROLE OF PHOSPHOLIPIDS IN *C. ELEGANS* AND OTHER NEMATODES

Phospholipids, such as phosphatidylglycerol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine, are major structural components of cellular membranes. In eukaryotes, phosphatidylcholine accounts for nearly 50% of total membrane phospholipids [39-40]. In *C. elegans*, the distribution of phospholipid headgroups is 55% ethanolamine, 32% choline, 8% sphingomyelin, and 5% other phospholipid classes, and this distribution changes in response to temperature [41-42]. In mammals, phosphatidylcholine is also a component in serum lipoproteins and can serve as a substrate or activator of phospholipases in signal transduction cascades [39-40].

As an adaptation to environmental conditions and growth in host organisms, as well as for structure and motility, nematodes produce an extracellular matrix composed of glycoproteins and carbohydrates [43]. The distribution of phosphorylcholine-substituted glycolipids changes in the extracellular matrix with different developmental stages of *C. elegans* [44-48]. Phosphorylcholine-substituted glycolipids are localized in seam cells and basement membranes during embryonic and post-embryonic stages, respectively, and the distribution shifts from lipid-bound to both lipid- and protein-bound forms as development progresses from late embryo to post-embryo [44].

Filarial nematode parasites avoid the host's immune system by modifying extracellular glycoproteins and glycolipids with phosphorylcholine derived from phosphatidylcholine [47]. Phosphorylcholine-containing antigens act as potent immunomodulatory molecules of *A. suum* (pig intestinal roundworm) [49-51]. Likewise, comparison of *C. elegans* and the human parasites *W.*

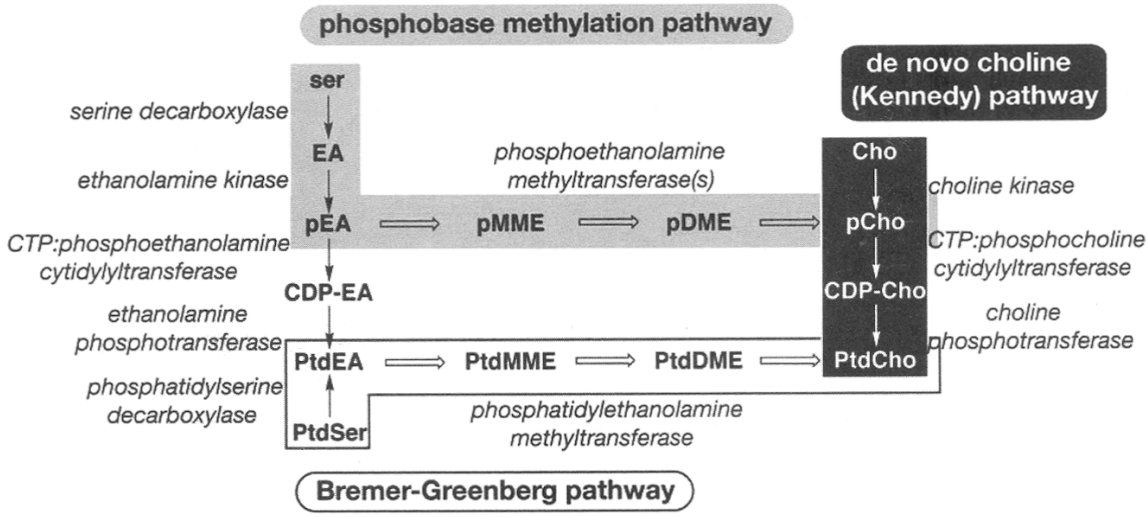

Fig. (1). Overview of phosphatidylcholine biosynthesis pathways. The metabolic steps of the de novo choline or Kennedy pathway (white on black), the Bremer-Greenberg pathway (black on white), and the plant-like phosphobase methylation pathway (black or gray) are shown. Conversions between the base, phosphobase, CDP, and phospholipid substrates are shown as vertical arrows. Horizontal arrows correspond to S-adenosylmethionine-dependent methylation reactions. Names of metabolites consist of a prefix (p: phospho; CDP-: cytidine 5'-diphosphate; or Ptd: phosphatidyl) and a core name (EA: ethanolamine; MME: monomethylethanolamine; DME: dimethylethanolamine; Cho: choline).

bancrofti, *B. malayi*, and *O. volvulus* show these organisms share phosphorylcholine-substituted oligosaccharides with common chemical structures [47, 52-53].

Studies are in the early stages but phospholipid analogues show promise against protozoa and trypanosomatids parasites [54-57]. Originally developed as anti-cancer drugs, phospholipid analogues, such as miltefosine (hexadecylphosphocholine), are active treatments for leishmaniasis [55]; however, the targets of action in the protozoa remain undefined. Nonetheless, such studies suggest the value of re-examining existing chemical libraries for anthelmintics or nematicides and of synthesizing molecules targeting enzymes in parasite pathways that differ from those of the host organism.

OVERVIEW OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS

The metabolic routes to phosphatidylcholine in mammals and higher eukaryotes are well studied [39-40, 58]. The core pathway of phosphatidylcholine biosynthesis is the *de novo* choline or Kennedy pathway, which consists of three enzymes that convert choline to phosphatidylcholine (Fig. (**1**)) [59-60]. A second pathway (i.e., the Bremer-Greenberg pathway) involves the sequential methylation of phosphatidylethanolamine to phosphatidylcholine (Fig. (**1**)) [61-62]. This route primarily occurs in yeast and in the mammalian liver, where production and secretion of bile and very low-density lipoprotein place increased demand on phosphatidylcholine production [63]. In plants, nearly all the metabolic flux into the Kennedy pathway occurs through a third biosynthetic route that involves the methylation of phosphoethanolamine to phosphocholine (Fig. (**1**)) [64-66].

As demonstrated for *Dirofilaria immitis* [67] and *C. elegans* [68-69], nematodes catalyze the reactions of both the Kennedy and Bremer-Greenberg pathway. Interestingly, the parasitic trematodes *Fasciola hepatica* and *Schistosoma mansoni* appear to only use the de novo choline pathway for phosphatidylcholine biosynthesis [70-71]. Recent publications suggest that the protozoan parasite *P.*

Table 2. ***C. Elegans*** **Genes and Enzymes in the** ***De Novo*** **Choline Pathway**

Name	Sequence	CGC	Confirmation	RNAi Phenotypes [Ref]
CK	C28D4.2	*cka-1*	cDNA/protein	none [29-30,81]
CK	C52B9.1a/b	*cka-2*	cDNA/protein	none [29-30,81]
CK	B0285.8	*ckb-1*	cDNA	reduced fat [31] embryo dev. [82] post-embryo dev. [30,81-82] none [29-30,81-82]
CK	B0285.9	*ckb-2*	cDNA/protein	maternal sterile [29-30,83] embryo lethal [29-30,83] embryo dev. [82] post-embryo dev. [30,81-82] none [29-30,81-82]
CK	B0285.10	*ckb-3*	predicted	none [29-30,81]
CK	F22F7.5	*ckb-4*	cDNA/protein	none [29-30,81]
CK	T27A.10.3a.1/2/b	*ckc-1*	cDNA	none [29-30,81]
CCT	F08C6.2a/b		cDNA/protein	larva arrest [29-30, 84] locomotion [84] none [29-30,81,85]
CCT	Y18H1A.11		partial cDNA	none [85]
CCT(?)	F28A10.10		predicted	none [29-30]
CCT(ECT?)	C39D10.3a/b		partial cDNA	none [29-30,81]
CCT(ECT?)	Y37E3.11		cDNA	none [29,85]
CPT	F22E10.5		partial cDNA	none [29-30]
CPT	F54D7.2		partial cDNA	slow growth [84] larva arrest [86] none [29,81]
CPT	Y49A3A.1		partial cDNA	embryo lethal [29-30,84] reduced fat content [31] long body [30] none [29]

Name: CK: choline kinase; CCT: CTP: phosphocholine cytidylyltransferase; ECT, CTP: phosphoethanolamine cytidylyltransferase; CPT, diacylglycerol cholinephosphotransferase. Sequences correspond to entries in WormBase. The assigned Caenorhabditis Genetics Center (CGC) name is noted. Confirmation indicates if the gene is either predicted from genome sequence, a partial cDNA isolated, a cDNA isolated, or the encoded protein expressed and assayed. RNAi phenotypes notes abnormal effects.

falciparum and the free-living nematode *C. elegans* also synthesize phosphocholine for incorporation into the Kennedy pathway using a plant-like phosphobase pathway [72-74]. Importantly, these metabolic variations between nematodes (and Plasmodium) and other organisms suggest that the different routes for phosphatidylcholine biosynthesis may be potential inhibitor targets.

THE *DE NOVO* CHOLINE OR KENNEDY PATHWAY IN *C. ELEGANS*

The *de novo* choline pathway consists of choline kinase (EC 2.7.1.32), CTP:phophosphocholine cytidylyltransferase (CCT, EC 2.7.7.15), and diacylglycerol cholinephosphotransferase (CPT, EC 2.7.8.2) (Fig. (**1**)). In *C. elegans*, choline kinase and CCT are the best-studied enzymes of the pathway [40, 75-78].

Choline kinase catalyzes the first step in this pathway using ATP as a phosphate donor to convert choline to phosphocholine. *C. elegans* encodes seven isoforms of this enzyme, which by sequence analysis cluster into three families A, B, and C (Table **2**) [75]. The A-family isoforms (*cka*-1 and -2)[1] are most similar to mammalian choline kinases (~35% identity) while the B-family (*ckb*-1, -2, -3, and -4) and C-family (*ckc*-1) are less similar (<25% identity) [75]. Expression of different isoforms is a likely mechanism for regulating choline kinase activity, but the physiologic role of each isoform remains undefined. Gee and Kent [75] cloned, expressed, and assayed four of the seven choline kinases (CKA-1, CKA-2, CKB-2, and CKB-4) from *C. elegans*. These four isoforms were 10- to 20-fold more active with choline than with ethanolamine as a substrate. Although most choline kinases accept either substrate, and ethanolamine-specific kinases have been isolated [79-80], it is unclear if any of the three biochemically uncharacterized choline kinase isoforms in *C. elegans* prefer ethanolamine as a substrate. Purification and kinetic analysis of CKA-2 (k_{cat} = 74 s^{-1}; $K_m^{choline}$ = 1.6 mM, K_m^{ATP} = 2.4 mM) and CKB-2 (k_{cat} = 3.8 s^{-1}; $K_m^{choline}$ = 13 mM, K_m^{ATP} = 0.7 mM) showed that the B-form was more active than the A-form. These functional studies set the stage for subsequent structural examination of the enzyme.

Peisach *et al.* [76] solved the x-ray crystal structure of *C. elegans* CKA-2 at 2.0 Å resolution (Fig. (**2**)). Each monomer of this homodimeric enzyme shares a similar fold with protein kinases and aminoglycoside phosphotransferases. Sequence alignments of choline/ethanolamine kinases from various species reveal two conserved amino acid sequence motifs [76]. The first region, known as Brenner's motif, occurs in many enzymes catalyzing phosphotransfer reactions. The second region is specifically conserved in the choline/ethanolamine kinases. Although crystallized in the absence of any ligands, comparison of the structure with protein kinases suggested that ATP binds in a pocket formed by these two conserved motifs and a third loop near the putative active site. Subsequent site-directed mutagenesis identified amino acids critical for catalysis in the Brenner's motif and the choline kinase motif [77]. In particular, Asp255, Asn260, and Asp301 were found to be important for ATP binding and Glu303 was required for activity, but an exact role for this residue is unclear [77]. The structure also suggested that two loops near these catalytically essential residues form a potential choline-binding site, but no additional information is available.

CTP:phophosphocholine cytidylyltransferase (CCT) catalyzes the synthesis of CDP-choline from CTP and phosphocholine and is a key regulatory enzyme [39-40]. The genome of *C. elegans* contains five genes sharing roughly 60% sequence identity with mammalian CCT (Table **2**). To date, only the gene product of F08C6.2 is confirmed as CCT [78]. This protein was expressed in baculovirus, purified, and biochemically characterized. Interestingly, the catalytic activity of the enzyme was enhanced 37-fold by phosphatidylcholine:oleate (1:1) vesicles. Further experiments mapped the lipid-activation domain to residues 246-266 and suggested that this domain is inhibitory to catalysis in the absence of lipids [78].

Sequence comparison of the putative CCT genes in *C. elegans* reveals both conserved and varied domain structures (Fig. (**3**)). Each gene contains a canonical CCT-like catalytic domain, which is similar in amino acid sequence to bacterial CTP:glycerol-3-phosphate cytidylyltransferase [78]. The lipid activation domain, as mapped in F08C6.2, is also present in Y18H1A.11, but this gene lacks the C-terminal phosphorylation region. The remaining three potential CCT genes are significantly different. The F28A10.10 gene, which has not been confirmed by cDNA isolation, encodes a stop codon after the CCT-like catalytic domain. It is unclear if this gene is a functional CCT lacking regulation or if it encodes a protein with a different enzymatic activity. Each of the two remaining genes (C39D10.3 and Y37E3.11) encode a CCT-like domain but sequence searching reveals greater similarity with CTP:ethanolamine-phosphate cytidylyltransferase [78], which may be involved in the pathway that transforms ethanolamine to phosphatidylethanolamine.

In *C. elegans*, three genes encode diacylglycerol cholinephosphotransferase, which catalyzes the conversion of CDP-choline and 1,2-diacylglycerol to CMP and phosphatidylcholine (Table **2**). It is tightly associated with the endoplasmic reticulum, is thought to be a seven transmembrane domain protein, and has not been purified to homogeneity from any source [39]. Similarly, there are no reports describing this enzyme or its activity in *C. elegans*.

Genome-wide RNAi experiments indicate that certain isoforms of the enzymes in the de novo choline pathway are needed for normal growth and development (Table **2**) [29-30,81-86]. Abnormal embryo and post-embryo developmental phenotypes were observed for *ckb*-1 and *ckb*-2 in some studies [30, 81-82]; however, most of the genome-wide experiments show little effect resulting from silencing of various choline kinase genes. This may result from functional redundancy of the isoforms or alternate routes for phosphatidylcholine synthesis. Of the CCT isoforms, only RNAi of F08C6.2 showed observed effects, i.e., larva arrest in some experiments [29-30, 84], but not in others [29-30, 81, 85]. These differences may result from variations in experimental design or worm growth conditions. Likewise, silencing of two diacylglycerol cholinephosphotransferase

[1]According to the guidelines for gene and protein nomenclature for *C. elegans* (http://www.cbs.umn.edu/CGC/Nomenclature/nomenguid.htm), genes are designated in italicized lower-case font and the encoded protein named in upper-case font.

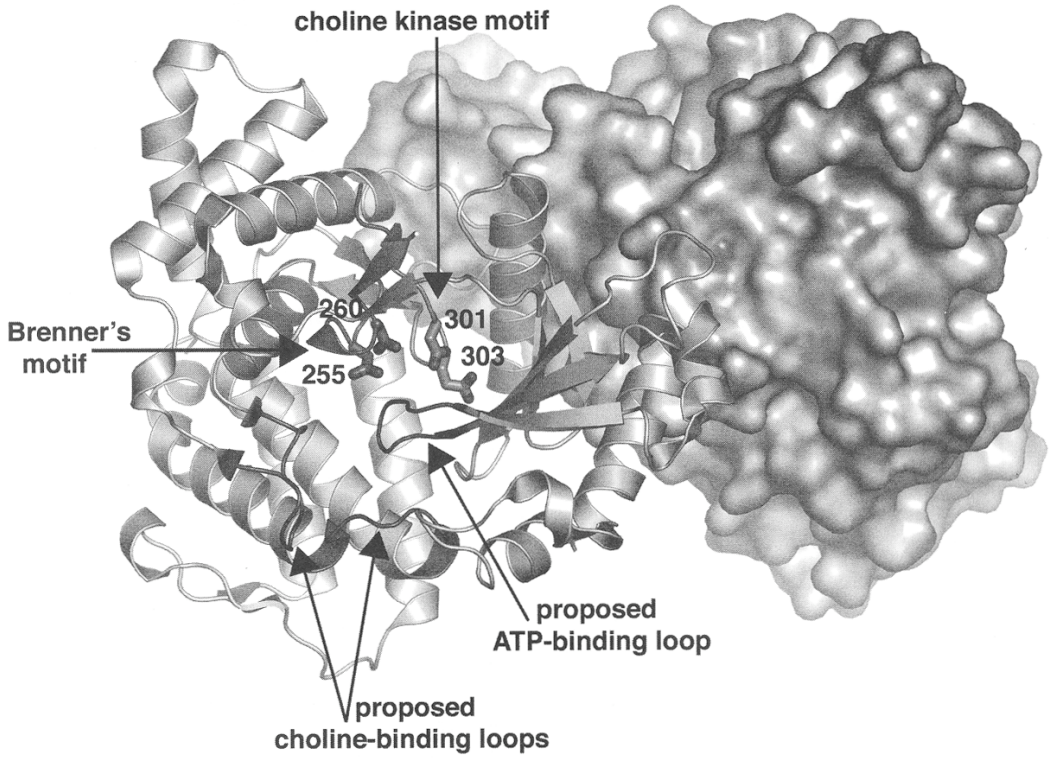

Fig. (2). Three-dimensional structure of choline kinase from *C. elegans*. The choline kinase dimer is shown with one monomer depicted as a ribbon diagram and the second monomer drawn as a surface rendering. Regions corresponding to Brenner's motif the choline kinase motif, the proposed ATP-binding loop and the proposed choline-binding loops are indicated. Catalytically important residues (Asp255, Asn260, Asp301, and Glu303) are shown as sticks. Figure created with Pymol (www.pymol.org).

isoforms results in phenotypes ranging from the arrest of larva growth to embryo lethality [29-31, 84, 86]. It should be noted that genome-wide RNAi screens are only the initial attempts to broadly define a role for these genes, supporting studies are required to elucidate the contribution of various enzymes in the Kennedy pathway of nematodes.

THE BREMER-GREENBERG PATHWAY IN *C. ELEGANS*

In mammals, dietary intake or lipid recycling provides choline for the Kennedy pathway, whereas, the Bremer-Greenberg pathway is the only endogenous route for phosphatidylcholine biosynthesis in humans and other

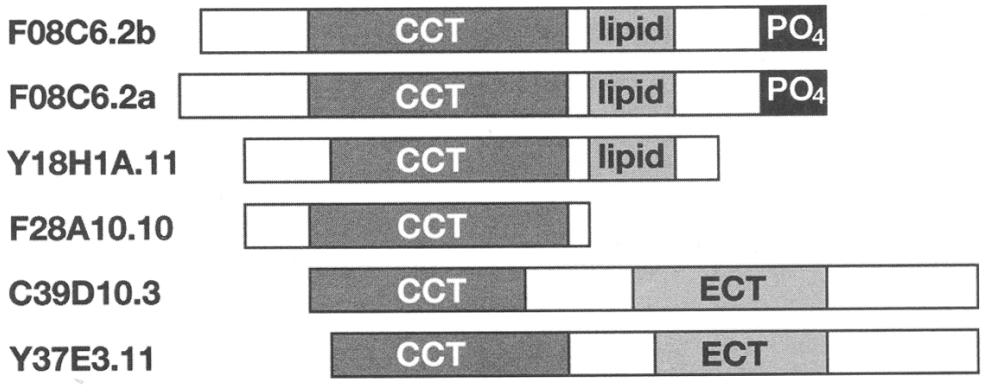

Fig. (3). Schematic of the domain structure of putative CTP:phosphocholine cytidylyltransferase from *C. elegans*. The regions encoding CTP:phosphocholine cytidylyltransferase (CCT)-like catalytic, lipid activation (lipid), phosphorylation (PO_4), and CTP:phosphoethanolamine cytidylyltransferase (ECT) domains are indicated. Size of the rectangle for each enzyme corresponds to amino acid length.

Table 3. *C. Elegans* **Genes and Enzymes in the Bremer-Greenberg Pathway**

Name	Sequence	CGC	Confirmation	RNAi Phenotypes [Ref]
PSD	B0361.5a/b	*psd*-1	partial cDNA	slow growth [30,84] embryo dev. [82] post-embryo dev. [81-82] clear [30] none [29,81-82]
PtdMT	F53B3.2.1/2		partial cDNA	none [29-30,81]
PtdMT	Y40B10A.2/6/7		partial cDNA	none [29-30,81]

Name: PSD, phosphatidylserine decarboxylase; PtdMT, phosphatidylethanolamine methyltransferase. Sequences correspond to entries in WormBase. The assigned Caenorhabditis Genetics Center (CGC) name is noted. Confirmation indicates if the gene is either predicted from genome sequence, a partial cDNA isolated, a cDNA isolated, or the encoded protein expressed and assayed. RNAi phenotypes notes abnormal effects.

mammals. In yeast and other fungi, the Bremer-Greenberg pathway serves as the primary route for phosphatidylcholine biosynthesis [87]. The physiologic contribution of this pathway to phosphatidylcholine synthesis in nematodes is unclear; however, the presence of genes encoding phosphatidylserine decarboxylase (EC 4.1.1.65) and phosphatidylethanolamine *N*-methyltransferase (EC 2.1.1.17) (Table **3**) in *C. elegans* implies a functional Bremer-Greenberg pathway (Fig. (**1**)).

The first reaction in the pathway converts phosphatidylserine to phosphatidylethanolamine and CO_2. The putative *C. elegans* phosphatidylserine decarboxylase (B0361.5a/b) shares less than 40% identity with the mammalian enzyme. In mice, disruption of the gene encoding the enzyme results in lethality during embryo development suggesting that metabolism of ethanolamine to phosphatidylethanolamine cannot substitute for phosphatidylserine decarboxylase during development [88]. Even though no information is available for the nematode gene or enzyme, RNAi screens yield results similar to those observed with transgenic mice, i.e., abnormal development at the embryo and post-embryo stages (Table **3**) [30, 81-82, 84].

Next, through a series of *S*-adenosylmethionine(SAM)-dependent reactions, phosphatidylethanolamine *N*-methyltransferase converts phosphatidylethanolamine to phosphatidylcholine. As with the first enzyme of the pathway, there are no published studies on the methyltransferases in *C. elegans*; however, the enzyme from humans has been biochemically isolated and studied. Although it is a transmembrane protein, assays of micelle preparations allowed for determination of the kinetic mechanism [89-90]. Conflicting kinetic models (random versus ordered bi bi sequential mechanisms) were reported but experiments show that a single enzyme catalyzes successive methylation reactions. Subsequent studies identified SAM-binding motifs and topologically mapped four membrane-spanning regions of the protein [91-92].

Gene silencing of either phosphatidylethanolamine *N*-methyltransferase gene in *C. elegans* (Table **3**) yields no observable phenotype [29-30,81]. It is unclear if these results indicate that the methylation of phosphatidylethanolamine is not essential or if other *C. elegans* pathways compensate for loss of these enzymes. Interestingly, disruption of the phosphatidylethanolamine *N*-methyltransferase gene in mice results in animals displaying no abnormal phenotype, normal hepatocyte morphology, normal plasmid lipid levels, and no differences in bile composition [93]. Later studies of mice undergoing severe choline deprivation exhibit increased choline kinase and CCT activity, but are able to survive

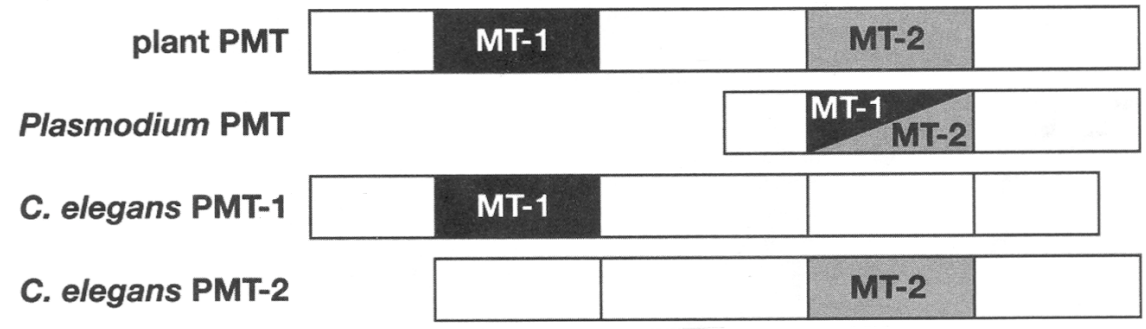

Fig. (4). Overview of the phosphoethanolamine methyltransferases (PMT) from plants, *P. falciparum*, and *C. elegans*. Methylation of phosphoethanolamine to phospho-monomethylethanolamine (P-MME) is catalyzed by the first methyltransferase domain (MT-1; white on black) and the next two methylation reactions that convert P-MME to phosphocholine are catalyzed by the second methyltransferase domain (MT-2; black on gray). The *C. elegans* PMT contain either the first (PMT-1) or second (PMT-2) methyltransferase domain. The empty boxes in the *C. elegans* PMT indicate a loss of the other catalytic domains. The *Plasmodium* PMT uses a single domain to perform all three methylation reactions. Size of the rectangle for each enzyme corresponds to amino acid length.

Table 4. ***C. Elegans*** **Genes and Enzymes in the Plant-Like Phosphobase Pathway**

Name	Sequence	CGC	Confirmation	RNAi Phenotypes [Ref]
PMT-1	ZK622.3a/b/c/d	*pmt-1*	cDNA/protein	embryo lethal [29-30,85] larva arrest [84-85] locomotion [84] slow growth [30] sluggish [30] none [31,81]
PMT-2	F54D11.1	*pmt-2*	cDNA/protein	larva arrest [74] sterility [74] lethality [74] none [29]

Name: PMT, phosphoethanolamine methyltransferase. Sequences correspond to entries in WormBase. The assigned Caenorhabditis Genetics Center (CGC) name is noted. Confirmation indicates if the gene is either predicted from genome sequence, a partial cDNA isolated, a cDNA isolated, or the encoded protein expressed and assayed. RNAi phenotypes notes abnormal effects.

complete choline deprivation by depleting hepatic phosphatidylcholine [94].

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Plants use a different pathway than mammals or fungi for the endogenous synthesis of phosphatidylcholine [64-66]. The phosphobase pathway of plants parallels the reactions of the Bremer-Greenberg pathway, as phosphoethanolamine methyltransferase (PMT, EC 2.1.1.103) methylates phosphoethanolamine to phosphocholine (Fig. (**1**)). In effect, this pathway bypasses the need for exogenous or recycled choline as a precursor for the Kennedy pathway.

The PMT from plants contain two methyltransferase domains with the N-terminal domain converting phosphoethanolamine to phospho-monomethylethanolamine (P-MME) and the C-terminal domain catalyzing the methylations of P-MME to phospho-dimethylethanolamine (P-DME) and P-DME to phosphocholine (Fig. (**4**)) [95]. A series of recent papers indicate that the protozoan parasite *P. falciparum* uses a plant-like phosphoethanolamine methylation pathway for phosphatidylcholine synthesis [72-73, 96]. In contrast to the plant enzymes, the *P. falciparum* PMT contains a single methyltransferase domain that performs all three methylation reactions (Fig. (**4**)) [72-73].

Two PMTs were originally identified in *C. elegans* from sequence comparisons performed with the plant and Plasmodium enzymes (Table **4**) [72-73,96]. Bioinformatic analysis shows that although related to the plant enzymes, the dual methyltransferase domain architecture is not found in the *C. elegans* PMT. The *C. elegans* genes encode either the N-terminal methyltransferase domain (*pmt*-1) or the C-terminal methyltransferase domain (*pmt*-2), even though both proteins are similar in size to the plant enzymes [74]. Biochemical characterization of *C. elegans* PMT-2 demonstrates that the protein catalyzes the methylations of P-MME to P-DME and of P-DME to phosphocholine using a random bi bi kinetic mechanism [74]. Similarly, unpublished studies indicate that PMT-1 only converts phosphoethanolamine to P-MME[2].

[2]Brendza K.M.; Haakenson, W.; Cahoon R.E.; Hicks, L.M.; Palavalli, L.H.; Chiapelli, B.J.; McLaird, M.; McCarter, J.P.; Williams, D.J.; Hresko, M.C.; Jez, J.M., manuscript in preparation.

Of the three metabolic routes to phosphatidylcholine in *C. elegans*, gene silencing of either *pmt*-1 or *pmt*-2 yield pronounced phenotypes (Table **4**). Genome-wide RNAi experiments show that silencing of *pmt*-1 causes embryo lethality, arrest of larva development, locomotion problems, and slow growth [29-30, 84-85]. More focused RNAi experiments show that PMT-2 is essential for worm viability and that choline supplementation, but not addition of ethanolamine, MME, or DME to growth media, rescues the RNAi-generated *pmt*-2 phenotype and restores normal growth and development [74].

Given the effect of PMT-targeted RNAi on the growth and development of *C. elegans*, these enzymes are interesting inhibitor targets. Both PMT-1 and PMT-2 are found in the genomes of multiple parasitic nematodes of mammals (*A. suum*, *Strongyloides stercoralis*, *H. contortus,* and *Ancylostoma ceylanicum*) and plants (*M. javanica*, *M. chitwoodi*, *M. incognita*, *Globodera rostochiensis*). Because the PMT are not found in the genomes of humans and animals, the development of molecules inhibiting the PMT may have medical and veterinary value. Although both plants and nematodes use the phosphobase methylation pathway for phosphatidylcholine biosynthesis, the nematode PMT are only distantly related (less than 25% sequence identity) to the plant homologs, which suggests that sufficient structural differences exist to facilitate identification of nematode-specific PMT inhibitors. Nonetheless, three-dimensional structures of plant and nematode PMT would further define these differences.

CONCLUSION

Identifying and characterizing new metabolic pathways or adaptations for parasitism raise the possibility of developing the next generation of nematicidal drugs with minimal side-effects and target specificity. As described in this review, phosphatidylcholine biosynthesis is a potential target for such efforts. Initial genome-wide RNAi screens and the first detailed RNAi studies indicate that multiple steps in the pathways leading to phosphatidylcholine are essential for normal growth and development of *C. elegans*; however, additional basic research is required to explore how the different biosynthetic routes leading to phospholipids function.

Biochemical studies have examined only a handful of the twenty proteins involved in phosphatidylcholine biosynthesis in *C. elegans*. Moreover, only minimal information is available on the specific metabolism of phospholipids in this worm. In mammals, the Bremer-Greenberg pathway is the only endogenous route to phosphatidylcholine, but in *C. elegans* this pathway and the plant-like phosphobase pathway serve as endogenous routes. In nematodes, the respective metabolic contributions of dietary intake of choline, lipid headgroup recycling, and endogenous biosynthesis to production of phosphatidylcholine remains an open question. Interestingly, RNAi experiments targeting PMT expression in *C. elegans* produces the most dramatic phenotypes, which suggests this pathway as the primary biosynthetic route in this organism.

Although *C. elegans* is an excellent platform for studying the general biology of nematodes, detailed knowledge about individual parasitic nematodes is required. Efforts to identify new targets will complement anthelmintic and nematicide discovery and development programs. Existing drug discovery programs may also yield new molecules, as the carry-over value of compounds developed for similar targets in other organisms may target parasitic nematodes. For example, the original development of phospholipid analogues for anti-cancer purposes yielded compounds with promise as anti-protozoal agents [54-57]. Likewise, compounds generated as potential inhibitors of human choline kinase in ras-related carcinogenesis [97] may have secondary value as anthelmintics or nematicides. Ultimately, the development of the next generation of molecules targeting parasitic nematodes will improve human, animal, and plant health worldwide.

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REFERENCES

[1] Chan, M.S. *Parasitol. Today*, **1997**, *13*, 438-443.
[2] Liu, L.X.; Weller, P.F. *New Engl. J. Med.*, **1996**, *334*, 1178-1184.
[3] Witty, M.J. *Int. J. Parasitol.*, **1999**, *29*, 95-103.
[4] Jasmer, D.P.; Goverse, A.; Smant, G. *Annu. Rev. Phytopathol.*, **2003**, *41*, 245-270.
[5] Sasser, J.N.; Freckman, D.W. In *Vistas on Nematology*; Veech, J.A.; Dickson, D.W., Eds.; Society of Nematology: Hyattsville, MD, **1987**, pp. 7-14.
[6] Davis, E.L.; Mitchum, M.G. *Plant Physiol.*, **2005**, *137*, 1182-1188.
[7] Chitwood, D.J. *Annu. Rev. Phytopathol.*, **2002**, *40*, 221-249.
[8] Kaminsky, R. *Curr. Opin. Infect. Dis.*, **2003**, *16*, 559-564.
[9] McKellar, Q.A.; Jackson, F. *Trends Parasitol.*, **2004**, *20*, 456-461.
[10] Omura, S.; Crump, A. *Nat. Rev. Microbiol.*, **2004**, *2*, 984-989.
[11] Jones, A.K.; Buckingham, S.D.; Sattelle, D.B. *Nat. Rev. Drug Discov.*, **2005**, *4*, 321-330.
[12] Williams, J.C. *Vet. Parasitol.*, **1997**, *72*, 461-470.
[13] Fischer, P.; Supali, T.; Maizels, R.M. *Trends Parasitol.*, **2004**, *20*, 351-355.
[14] Duncan, L.W. *Annu. Rev. Phytopathol.*, **1991**, *29*, 469-490.
[15] Jung, C.; Wyss, U. *Appl. Microbiol. Biotechnol.*, **1999**, *51*, 439-446.
[16] Bakhetia, M.; Charlton, W.L.; Urwin, P.E.; McPherson, M.J.; Atkinson, H.J. *Trends Plant Sci.*, **2005**, *10*, 363-367.
[17] Brenner, S. *Genetics*, **1974**, *77*, 71-94.
[18] Geary, T.G.; Thompson, D.P. *Vet. Parasitol.*, **2001**, *101*, 371-386.
[19] Hashmi, S.; Tawe, W.; Lustigman, S. *Trends Parasitiol.*, **2001**, *17*, 387-393.
[20] Kaletta, T.; Hengartner, M.O. *Nat. Rev. Drug Discov.*, **2006**, *5*, 387-398.
[21] Riddle, D.L.; Blumenthal, T.; Meyer, B.J.; Priess, J.R., Eds. *C. elegans II*, Cold Spring Harbor Laboratory Press, **1997**.
[22] Hope, I.A., Ed., *C. elegans: a practical approach*, Oxford University Press, **1999**.
[23] Sulston, J.E.; Horvitz, H.R. *Dev. Biol.*, **1977**, *56*, 110-156.
[24] The *C. elegans* Sequencing Consortium. *Science*, **1998**, *282*, 2012-2018.
[25] Schwarz, E.M.; Antoshechkin, I.; Bastiani, C.; Bieri, T.; Blasiar, D.; Canaran, P.; Chan, J.; Chen, N.; Chen, W.J.; Davis, P.; Fiedler, T.J.; Girard, L.; Harris, T.W.; Kenny, E.E.; Kishore, R.; Lawson, D.; Lee, R.; Muller, H.M.; Nakamura, C.; Ozersky, P.; Petcherski, A.; Rogers, A.; Spooner, W.; Tuli, M.A.; Van Auken, K.; Wang, D.; Durbin, R.; Spieth, J.; Stein, L.D.; Sternberg, P.W. *Nucleic Acids Res.*, **2006**, *34*, D475-478.
[26] Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. *Nature*, **1998**, *391*, 806-811.
[27] Tabara, H.; Grishok, A.; Mello, C.C. *Science*, **1998**, *282*, 430-431.
[28] Timmons, L.; Fire, A. *Nature*, **1998**, *395*, 854.
[29] Sonnichsen, B.; Koski, L.B.; Walsh, A.; Marschall, P.; Neumann, B.; Brehm, M.; Alleaume, A.M.; Artelt, J.; Bettencourt, P.; Cassin, E.; Hewitson, M.; Holz, C.; Khan, M.; Lazik, S.; Martin, C.; Nitzsche, B.; Ruer, M.; Stamford, J.; Winzi, M.; Heinkel, R.; Roder, M.; Finell, J.; Hantsch, H.; Jones, S.J.; Jones, M.; Piano, F.; Gunsalus, K.C.; Oegema, K.; Gonczy, P.; Coulson, A.; Hyman, A.A.; Echeverri, C.J. *Nature*, **2005**, *434*, 462-469.
[30] Kamath, R.S.; Fraser, A.G.; Dong, Y.; Poulin, G.; Durbin, R.; Gotta, M.; Kanapin, A.; Le Bot, N.; Moreno, S.; Sohrmann, M.; Welchman, D.P.; Zipperlen, P.; Ahringer, J. *Nature*, **2003**, *421*, 231-237.
[31] Ashrafi, K.; Chang, F.Y.; Watts, J.L.; Fraser, A.G.; Kamath, R.S.; Ahringer, J.; Ruvkun, G. *Nature*, **2003**, *421*, 268-272.
[32] Kim, J.K.; Gabel, H.W.; Kamath, R.S.; Tewari, M.; Pasquinelli, A.; Rual, J.F.; Kennedy, S.; Dybbs, M.; Bertin, N.; Kaplan, J.M.; Vidal, M.; Ruvkun, G. *Science*, **2005**, *308*, 1164-1167.
[33] Fleming, J.T.; Squire, M.D.; Barnes, T.M.; Tornoe, C.; Matsuda, K.; Ahnn, J.; Fire, A.; Sulston, J.E.; Barnard, E.A.; Sattelle, D.B.; Lewis, J.A. *J. Neurosci.*, **1997**, *15*, 5843-5857.
[34] McCarter, J.P. *Trends Parasitol.*, **2004**, *20*, 462-468.
[35] Behm, C.A.; Bendig, M.M.; McCarter, J.P.; Sluder, A.E. *Trends Parasitol.*, **2005**, *21*, 97-100.
[36] Mitreva, M.; Blaxter, M.L.; Bird, D.M.; McCarter, J.P. *Trends Genet.*, **2005**, *21*, 573-581.
[37] Hussein, A.S.; Kichenin, K.; Selkirk, M.E. *Mol. Biochem. Parasitol.*, **2002**, *122*, 91-94.
[38] Aboobaker, A.A.; Blaxter, M.L. *Mol. Biochem. Parasitol.*, **2003**, *129*, 41-51.
[39] Kent, C. *Annu. Rev. Biochem.*, **1995**, *64*, 315-343.
[40] Kent, C. *Biochim. Biophys. Acta*, **2005**, *1733*, 53-66.
[41] Satouchi, K.; Hirano, K.; Sakaguchi, M.; Takehara, H.; Matsuura, F. *Lipids*, **1993**, *28*, 837-840.
[42] Tanaka, T.; Ikita, K.; Ashida, T.; Motoyama, Y.; Yamaguchi, Y.; Satouchi, K. *Lipids*, **1996**, *31*, 1173-1178.
[43] Kramer, J.M. In *C. elegans II*; Riddle, D.L.; Blumenthal, T.; Meyer, B.J.; Priess, J.R., Eds.; Cold Spring Harbor Laboratory Press, **1997**; pp. 471-500.
[44] Gerdt, S.; Dennis, R.D.; Borgonie, G.; Schnabel, R.; Geyer, R. *Eur. J. Biochem.*, **1999**, *266*, 952-963.
[45] Tanaka, T.; Izuwa, S.; Tanaka, K.; Yamamoto, D.; Takimoto, T.; Matsuura, F.; Satouchi, K. *Eur. J. Biochem.*, **1999**, *263*, 189-194.
[46] Cipollo, J.F.; Costello, C.E.; Hirschberg, C.B. *J. Biol. Chem.*, **2002**, *277*, 49143-49157.
[47] Cipollo, J.F.; Awad, A.; Costello, C.E.; Robbins, P.W.; Hirschberg, C.B. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 3404-3408.
[48] Cipollo, J.F.; Awad, A.; Costello, C.E.; Hirschberg, C.B. *J. Biol. Chem.*, **2005**, *280*, 26063-26072.
[49] Houston, K.M.; Harnett, W. *Parasitol.*, **1999**, *118*, 311-318.

[50] Deehan, M.R.; Goodridge, H.S.; Blair, D.; Lochnit, G.; Dennis, R.D.; Geyer, R.; Harnett, M.M.; Harnett. W. *Parasite Immunol.*, **2002**, *24*, 463-469.
[51] Kean, D.E.; Ohtsuka, I.; Sato, K.; Hada, N.; Takeda, T.; Lochnit, G.; Geyer, R.; Harnett, M.M.; Harnett, W. *Parasite Immunol.*, **2006**, *28*, 69-76.
[52] Haslam, S.M.; Morris, H.R.; Dell, A. *Trends Parasitol.*, **2001**, *17*, 231-235.
[53] Haslam, S.M.; Houston, K.M.; Harnett, W.; Reason, A.J.; Morris, H.R.; Dell, A. *J. Biol. Chem.*, **1999**, *274*, 20953-20960.
[54] Vial, H.J.; Eldin, P.; Tielens, A.G.M.; van Hellemond, J.J. *Mol. Biochem. Parasitol.*, **2003**, *126*, 143-154.
[55] Croft, S.L.; Seifert, K.; Duchene, M. *Mol. Biochem. Parasiol.*, **2003**, *126*, 165-172.
[56] Roberts, C.W.; McLeod, R.; Rice, D.W.; Ginger, M.; Chance, M.L.; Goad, L.J. *Mol. Biochem. Parasitol.*, **2003**, *126*, 129-142.
[57] Chaudhary, K.; Roos, D.S. *Nat. Biotech.*, **2005**, *23*, 1089-1091.
[58] Vance, J.E.; Vance, D.E. *Biochem. Cell Biol.*, **2004**, *82*, 113-128.
[59] Kennedy, E.P.; Weiss, S.B. *J. Biol. Chem.*, **1956**, *222*, 193-213.
[60] Borkenhagen, L.F.; Kennedy, E.P. *J. Biol. Chem.*, **1957**, *227*, 951-962.
[61] Bremer, J.; Figard, P.H.; Greenberg, D.M. *Biochim. Biophys. Acta*, **1960**, *46*, 477-488.
[62] Bremer, J.; Greenberg, D.M. *Biochim. Biophys. Acta*, **1961**, *46*, 205-216.
[63] Vance, D.E.; Walkey, C.J.; Cui, Z. *Biochim. Biophys. Acta*, **1997**, *1348*, 142-150.
[64] Mudd, S.H.; Datko, A.H. *Plant Physiol.*, **1986**, *82*, 126-135.
[65] Datko, A.H.; Mudd, S.H. *Plant Physiol.*, **1988**, *88*, 854-861.
[66] Datko, A.H.; Mudd, S.H. *Plant Physiol.*, **1988**, *88*, 1338-1348.
[67] Srivastava, A.K.; Jaffe, J.J. *Int. J. Parasitol.*, **1985**, *15*, 27-31.
[68] Lochnit, G.; Geyer, R. *Acta Biochim. Pol.*, **2003**, *50*, 1239-1242.
[69] Lochnit, G.; Bongaarts, R.; Geyer, R. *Int. J. Parasiol.*, **2005**, *35*, 911-923.
[70] Oldenberg, V.; Vugt, F.; Golde, L.M. *Biochim. Biophys. Acta*, **1975**, *398*, 101-110.
[71] Young, B.W.; Podesta, R.B. *Mol. Biochem. Parasitol.*, **1982**, *5*, 165-172.
[72] Pessi, G.; Kociubinski, G.; Ben Mamoun, C. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 6206-6211.
[73] Pessi, G.; Choi, J.Y.; Reynolds, J.M.; Voelker, D.R.; Ben Mamoun, C. *J. Biol. Chem.*, **2005**, *280*, 12461-12466.
[74] Palavalli, L.H.; Brendza, K.M.; Haakenson, W.; Cahoon, R.E.; McLaird, M.; Hicks, L.M.; McCarter, J.P.; Williams, D.J.; Hresko, M.C.; Jez, J.M. *Biochemistry*, **2006**, *45*, 6056-6065.
[75] Gee, P.; Kent, C. *Biochim. Biophys. Acta*, **2003**, *1648*, 33-42.
[76] Peisach, D.; Gee, P.; Kent, C.; Xu, Z. *Structure*, **2003**, *11*, 703-713.
[77] Yuan, C.; Kent, C. *J. Biol. Chem.*, **2004**, *279*, 17801-17809.
[78] Friesen, J.A.; Liu, M.F., Kent, C. *Biochim. Biophys. Acta*, **2001**, *1533*, 86-98.
[79] Kim, K.; Kim, K.H.; Storey M.K.; Voelker, D.R.; Carman, G.M. *J. Biol. Chem.*, **1999**, *274*, 14857-14866.
[80] Lykidis, A.; Wang, J.; Karim, M.A.; Jackowski, S. *J. Biol. Chem.*, **2001**, *276*, 2174-2179.
[81] Rual, J.F.; Ceron, J.; Koreth, J.; Hao, T.; Nicot, A.S.; Hirozane-Kishikawa, T.; Vandenhaute, J.; Orkin, S.H.; Hill, D.E.; van den Heuvel, S.; Vidal, M. *Genome Res.*, **2004**, *14*, 2162-2168.
[82] Gonczy, P.; Echeverri, C.; Oegema, K.; Coulson, A.; Jones, S.J.; Copley, R.R.; Duperon, J.; Oegema, J.; Brehm, M.; Cassin, E.; Hannak, E.; Kirkham, M.; Pichler, S.; Flohrs, K.; Goessen, A.; Leidel, S.; Alleaume, A.M.; Martin, C.; Ozlu, N.; Bork, P.; Hyman, A.A. *Nature*, **2000**, *408*, 331-336.
[83] Piano, F.; Schetter, A.J.; Mangone, M.; Stein, L.; Kemphues, K.J. *Curr. Biol.*, **2000**, *10*, 1619-1622.
[84] Simmer, F.; Moorman, C.; van der Linden, A.M.; Kuijk, E.; van den Berghe, P.V.; Kamath, R.S.; Fraser, A.G.; Ahringer, J.; Plasterk, R.H. *PLoS Biol.*, **2003**, *1*, E12.
[85] Maeda, I.; Kohara, Y.; Yamamoto, M.; Sugimoto, A. *Curr. Biol.*, **2001**, *11*, 171-176.
[86] Fraser, A.G.; Kamath, R.S.; Zipperlen, P.; Martinez-Campos, M.; Sohrmann, M.; Ahringer, J. *Nature*, **2000**, *408*, 325-330.
[87] Kanipes, M.I.; Henry, S.A. *Biochim. Biophys. Acta*, **1997**, *1348*, 134-141.
[88] Steenbergen, R.; Nanowski, T.S.; Beigneux, A.; Kulinski, A.; Young, S.G.; Vance, J.E. *J. Biol. Chem.*, **2005**, *280*, 40032-40040.
[89] Ridgway, N.D.; Vance, D.E. *J. Biol. Chem.*, **1988**, *263*, 16864-16871.
[90] Reitz, R.C.; Mead, D.J.; Bjur, R.A.; Greenhouse, A.H.; Welch, W.H. *J. Biol. Chem.*, **1989**, *264*, 8097-8106.
[91] Shields, D.J.; Lehner, R.; Agellon, L.B.; Vance, D.E. *J. Biol. Chem.*, **2003**, *278*, 2956-2962.
[92] Shields, D.J.; Altarejos, J.Y.; Wang, X.; Agellon, L.B.; Vance, D.E. *J. Biol. Chem.*, **2003**, *278*, 35826-35836.
[93] Walkey, C.J.; Donohue, L.R.; Bronson, R.; Agellon, L.B.; Vance, D.E. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 12880-12885.
[94] Li, Z.; Agellon, L.B.; Vance, D.E. *J. Biol. Chem.*, **2005**, *280*, 37796-37802.
[95] Nuccio, M.L.; Ziemak, M.J.; Henry, S.A.; Weretilnyk, E.A.; Hanson, A.D. *J. Biol. Chem.*, **2000**, *275*, 14095-14101.
[96] Witola, W.H.; Pessi, G.; El-Bissati, K., Reynolds, J.M.; Ben Mamoun, C. *J. Biol. Chem.*, **2006**, *281*, 21305-21311.
[97] Janardhan, S.; Srivani, P.; Sastry, G.N. *Curr. Med. Chem.*, **2006**, *13*, 1169-1186.

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